PTO/SB/17 (07-06)

Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2006

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 500

Complete if Known	
Application Number	09/361,652
Filing Date	July 27, 1999
First Named Inventor	Zuker, Charles S.
Examiner Name	Michael T. Brannock
Art Unit	1649
Attorney Docket No.	02307E-088610US

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☒ Deposit Account Deposit Account Number: 20-1430 Deposit Account Name: Townsend and Townsend and Crew LLP

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

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FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fees Paid ($)
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims _____ -20 or HP = _____ x Fee ($) _____ = Fee Paid ($) _____

HP = highest number of total claims paid for, if greater than 20

Indep. Claims _____ -3 or HP = _____ x Fee ($) _____ = Fee Paid ($) _____

HP = highest number of independent claims paid for, if greater than 3

Multiple Dependent Claims Fee ($) _____ Fee Paid ($) _____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets _____ - 100 = Extra Sheets _____ / 50 = Number of each additional 50 or fraction thereof _____ (round up to a whole number) x Fee ($) _____ = Fee Paid ($) _____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): Filing a brief in support of an appeal	500

SUBMITTED BY

Signature		Registration No. (Attorney/Agent) 54,111	Telephone 415-576-0200
Name (Print/Type)	Chuan Gao		Date February 2, 2007

11156686 v1

PATENT
Atty. Docket No.: 02307E-088610US
DHHS Ref. No. E-003-99/0
U.C. Ref. No. 98-306-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 5785
Zuker et al.	Examiner: Michael Brannock
Application No.: 09/361,652	Technology Center/Art Unit: 1646
Filed: July 27, 1999	
For: NUCLEIC ACIDS ENCODING A G-PROTEIN COUPLED RECEPTOR INVOLVED IN SENSORY TRANSDUCTION	APPELLANT'S BRIEF UNDER 37 C.F.R. §43.17
Customer No.: 20350	

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This brief is filed pursuant to 37 C.F.R. §43.17, following the Notice of Appeal filed on September 18, 2006. A petition to extend time to submit the Appeal Brief for three months, from November 18, 2006, to February 18, 2007, is filed herewith. Also submitted with this brief is authorization to pay the fee as set forth in 37 C.F.R. §1.17(c).

TABLE OF CONTENTS

I. REAL PARTY IN INTEREST

The real party in interest in U.S. Application No. 09/361,652 is the Regents of the University of California and the National Institutes of Health.

II. RELATED APPEALS AND INTERFERENCES

There are no other pending appeals by Appellants or interferences in which Appellants are involved the outcome of which would directly affect the decision by the Board of Patent Appeals and Interferences in this pending appeal.

III. STATUS OF THE CLAIMS

Claims 1-63 were originally filed. Subsequently, claims 2, 3, 7, 9-33, 36-60 were canceled and claims 64-67 were added. Claims 1, 4-6, 8, 34, 35, and 61-67 are pending in this application. In the final Office Action mailed July 13, 2006, the Examiner rejects all pending claims (1, 4-6, 8, 34, 35, and 61-67) under 35 U.S.C. §101, alleging lack of either a credible specific and substantial utility, or a well-established utility. The Examiner also rejects claims 1, 4-6, 8, 34, 35, and 61-67 under 35 U.S.C. §112, first paragraph, alleging failure to enable the claimed invention based on the utility rejection. Furthermore, the Examiner rejects claims 1, 6, 34, 35, and 61-67 under 35 U.S.C. §112, first paragraph, for alleged inadequate written description. The rejections of claims 1, 4-6, 8, 34, 35, and 61-67 are being appealed.

IV. STATUS OF THE AMENDMENTS

No amendment was filed subsequent to the final Office Action of July 13, 2006.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The claimed subject matter in this appeal relates to a nucleic acid encoding a taste transduction G-protein coupled receptor (GPCR), as well as methods for making an expression vector and a recombinant cell for producing the GPCR recombinantly.

Claim 1

The subject matter claimed in independent claim 1 is an isolated nucleic acid encoding a taste transduction G-protein coupled receptor. The receptor comprises an amino acid sequence having at least 80% identity to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, and binds to glutamate, which induces GPCR activity.

Claim 61

The subject matter claimed in independent claim 61 is a method of making a taste transduction G-protein coupled receptor. The method comprises the step of expressing the receptor from a recombinant expression vector comprising a nucleic acid encoding the receptor, wherein the receptor comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, and binds glutamate, which induces GPCR activity.

Claim 62

The subject matter claimed in independent claim 62 is a method of making a recombinant cell comprising a taste transduction G-protein coupled receptor. The method comprising the step of transducing the cell with an expression vector comprising a nucleic acid encoding the receptor, wherein the receptor comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, and wherein the receptor binds glutamate, which induces GPCR activity.

Claim 63

The subject matter claimed in independent claim 63 is a method of making an recombinant expression vector comprising a nucleic acid encoding a taste transduction G-protein coupled receptor. The method comprises the step of ligating to an expression vector a nucleic acid encoding the receptor, wherein the receptor comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, and wherein the receptor binds glutamate, which induces GPCR activity.

VI. GROUNDS OF REJECTION TO BE REVIEWED AND APPEALED

1. Claims 1, 4-6, 8, 34, 35, and 61-67 stand rejected for alleged lack of utility.

2. Claims 1, 4-6, 8, 34, 35, and 61-67 stand rejected for alleged lack of enablement.

3. Claims 1, 6, 34, and 61-67 stand rejected for alleged lack of written description.

VII. ARGUMENT

A. The Rejection for Lack of Utility Is Improper

Claims 1, 4-6, 8, 34, 35, and 61-67 stand rejected under 35 U.S.C. §101 because the Examiner alleges that the claimed invention lacks either a well-established utility or a credible specific and substantial asserted utility. Appellants respectfully traverse this rejection and argue that the rejection is improper.

1. Standard to Assess Utility

According to MPEP §2107, the Examiner should review the claims and the supporting written description to determine whether the utility requirement under 35 U.S.C. §101 is met. No rejection based on lack of utility should be made if an invention has a well-established utility, *i.e.*, a utility that will be immediately appreciated by one of ordinary skill in the art based on the characteristics of the invention, regardless any such utility has been asserted. Neither should any rejection be made for lack of utility if an applicant has asserted a specific and substantial utility that would be considered credible by one of ordinary skill in the art.

In most cases, an applicant's assertion of utility creates a presumption of utility that will be sufficient to satisfy the utility requirement of 35 U.S.C. §101. MPEP §2107.02 III A. The Court of Customs and Patent Appeals stated in *In re Langer*:

> As a matter of Patent Office practice, a specification which contains a disclosure of utility which corresponds in scope to the subject matter sought to be patented must be taken as sufficient to satisfy the utility requirement of §101 for the entire claimed subject matter unless there is a reason for one skilled in the art to question the objective truth of the statement of utility or its scope.

In re Langer, 183 USPQ 288, 297 (CCPA, 1974, emphasis in original). To overcome the presumption of sufficient utility as asserted by an applicant, the Examiner must carry the initial burden to make a *prima facie* showing of lack of utility and provide a sufficient evidentiary basis for the conclusion. In other words, the Examiner "must do more than merely question operability--[he] must set forth factual reasons which would lead one skilled in the art to question objective truth of the statement of operability." *In re Gaubert*, 187 USPQ 664, 666 (CCPA 1975).

MPEP §2107.02 IV further states, a detailed explanation should be given for a utility rejection as to why the claimed invention has no specific and substantial asserted utility. Documentary evidence should be provided when possible. Otherwise the Examiner should specifically explain the scientific basis for his factual conclusions.

Moreover, the MPEP states that once the examiner presents a *prima facie* case of unpatentability for lack of utility, the burden of coming forth with evidence or arguments shifts to the applicant. After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record and by a preponderance of evidence with due consideration to persuasiveness of argument. "If the record as a whole would make it more likely than not that the asserted utility for the claimed invention would be considered credible by a person of ordinary skill in the art, the Office cannot maintain the rejection." MPEP §2107.02 VI.

2. The Asserted Utility and the Examiner's Rejection

The present invention relates to the identification of G-protein coupled receptor (GPCR) B3, a GPCR expressed specifically in taste cells. It is asserted in the specification that this taste cell specific GPCR is a component of the taste signal

transduction pathway and is capable of, via its interaction with a G-protein, mediating taste (such as sweet, bitter, unami, *etc.*) perception. *See, e.g.*, page 3, lines 7-10; page 3, line 31, to page 4, line 2; and page 9, lines 30-33, of the specification. It is further asserted that GPCR-B3 polypeptides or the encoding nucleic acids can be used, for example, as probes to identify taste cells, to generate taste topographic map, and to provide a screening method for compounds that can modulate taste signaling and are therefore useful in the food and pharmaceutical industries. *See, e.g.*, page 8, line 16, to page 9, line 10, of the specification.

In addition, Appellants previously submitted Dr. Zuker's declaration under 37 C.F.R. §1.132 (along with Appellants' response of September 16, 2002). In this declaration, Dr. Zuker attests that given the structure and expression pattern of GPCR-B3, as well as the results of a functional assay using a chimeric GPCR construct, one of skill in the art would readily recognize, at the time this application was filed, the immediately available of use of the claimed GPCR-B3 polynucleotides or polypeptides, *e.g.*, for identifying modulators of taste signal transduction. It is therefore established that an ordinarily skilled artisan would find the asserted utility specific, substantial, and credible.

In several previous Office Actions as well as the July 13, 2006, final Office Action, the Examiner takes the position that the GPRC-B3 polypeptide lacks substantial utility, or a "real world" use, because the polypeptide is not described as to be involved in any particular aspect of the taste perception. In response, Appellants cited in their response of October 23, 2003, the reference by Nelson *et al.* (attached as Appendix 2 of this brief, previously made of record as reference BE in the IDS filed September 16, 2002), which shows that GPCR-B3 (also known as T1R1), forming a heterodimeric GPCR with T1R3 and functioning as an L-amino acid taste receptor, is indeed involved in a definitive aspect of the taste perception. In the previous Office Actions and the July 13, 2006, final Office Action, however, the Examiner continues to disagree with Appellants' position that the Nelson *et al.* reference supports a finding of patentable

utility, stating that since the presence of GPCR-B3 in this heterodimeric receptor is described only in this reference but not in the present specification, one of skill in the art would not recognize or believe such use of GPCR-B3 after reading the specification.

3. The Examiner Has Raised and Maintained the Utility Rejection in a Manner Inconsistent with the MPEP

Appellants have asserted a specific and substantial utility in the specification and submitted Dr. Zuker's declaration to demonstrate that this asserted utility is credible to one of skill in the art. In contrast, the Examiner has not provided any evidence or objective reason to overcome the presumed patentable utility. The Nelson *et al.* reference was provided merely as an example of confirmed involvement of GPCR-B3 in taste signaling. On the other hand, it is possible that GPCR-B3 can act alone or in complex with other proteins including other GPCRs to mediate taste signal transduction. The Nelson reference was not cited by any means to indicate or suggest that GPCR-B3's sole involvement in taste perception is via complexing with T1R3 to form a heterodimer. This reference was cited to demonstrate the credibility of the asserted utility that GPCR-B3 is involved in taste signaling and is therefore useful in, *e.g.*, screening methods for identifying taste-modulating compounds. Thus, whether or not the specification describes this particular heterodimer of GPCR-B3 and T1R3 is not directly relevant to whether one of skill in the art would find the asserted utility credible. In fact, the notion that one of skill in the art would, at the time this application was filed, find the asserted utility (which is not limited to the heterodimer of GPCR-B3 and T1R3) credible has already been established by Dr. Zuker's declaration and not yet rebutted by the Examiner.

Raising and maintaining a rejection for lack of utility in such a manner is inconsistent with the proper practice described in the MPEP, which places the initial burden on the Examiner, not Appellants, to provide evidence to support a factual conclusion of the credibility of an asserted utility. In fact, MPEP §2107.02 III.B. specifically cautions Office personnel that, once an assertion of a particular utility is

made, "that assertion cannot simply be dismissed ..... as 'wrong,' even when there may be reason to believe the assertion is not entirely accurate." Instead, the Examiner must provide an explanation setting forth the reasoning used in concluding that the asserted specific and substantial utility is not credible; support for factual findings relied upon in reaching the conclusion; and an evaluation of all relevant evidence of record, including utilities taught in the closest prior art. MPEP §2107.02 IV. Furthermore, it is stated in MPEP §2107.02 VI that, after the Examiner has provided evidence or objective reasons to question the credibility of an asserted utility, and the applicant has further responded with evidence or argument, "patentability is determined on the totality of the record, by a preponderance of evidence with due consideration to persuasiveness of argument." A utility rejection cannot be properly maintained if the record as a whole indicates it is more likely than not that one of skill in the art would consider the asserted utility credible.

In the present case, Appellants have responded to the Examiner's questioning of the asserted utility by offering Dr. Zuker's declaration, which establishes that one of skill in the art would reasonably believe the asserted utility, as well as the Nelson *et al.* reference, which provides an example of GPCR-B3's involvement in taste signaling; on the other hand, the Examiner has offered no evidence or objective reasons why the asserted utility is not credible. Indeed, in the final Office Action of July 13, 2006, the Examiner again rejects the Appellants' assertion of utility, particular the assertion that GPCR-B3 alone can act alone or in complex with other proteins to mediate taste signaling, stating that "[m]any things are possible but simply inviting an artisan to test various ideas to try to find a way to use the polypeptide does not provide for a substantial utility" (the first full paragraph on page 3 of the final Office Action). Offering no evidence or objective reasons to contradict the asserted utility, the Examiner's statements merely reflect his personal disbelief of the asserted utility, which is supported by both Dr. Zuker's declaration and the Nelson reference.

Appellants thus submit that when considered together, the record favors a holding of sufficient credibility in the asserted utility.

4. Summary

Appellants do not believe that the Examiner has adhered to the proper standards for assessing utility as described in the MPEP. The utility rejection is therefore improper and should be withdrawn.

B. The Rejection for Inadequate Enablement Based on Utility Is Improper

The Examiner has also maintained the rejection of claims 1, 4-6, 34, 35, and 61-67 on enablement ground, alleging that the claimed invention is not supported by either a credible specific and substantial asserted utility or a well-established utility. As discussed above, the claimed invention has a credible specific and substantial utility. Appellants therefore believe that the enablement rejection under 35 U.S.C. §112, first paragraph, is improper and should be withdrawn.

C. The Rejection for Inadequate Written Description Is Improper

The Examiner has further rejected claims 1, 6, 34, 35, and 61-67 under 35 U.S.C. §112, first paragraph, alleging that the claimed invention is not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention.

As will be discussed in detail below, the claimed invention as described in the present application fully complies with the requirement for written description as set forth by the MPEP and prevailing case law. Appellants thus submit that the written description rejection is improper and should be withdrawn.

1. Standard for Written Description

According to the MPEP, to satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail such that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. Possession of a claimed invention may be demonstrated by description of the invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention. MPEP §2163 I. Moreover, a strong presumption exists with regard to originally filed claims that an adequate written description of the claimed invention is present when the application is filed. MPEP §2163 I.A.

Case law indicates that structural features of a claimed invention are important for satisfying the written description requirement. The Federal Circuit in *Fiers v. Revel*, 25 USPQ2d 1601 (Fed. Cir. 1993), stated that an adequate written description "requires a precise definition, such as by structure, formula, chemical name, or physical properties." *Fiers*, 25 USPQ2d at 1606. The requirement for written description of a chemical genus is further set forth in *University of California v. Eli Lilly & Co.*, 43 USPQ2d 1398 (Fed. Cir. 1997). As described by the Federal Circuit in *Lilly*, "[a] description of a genus of cDNAs may be achieved by means of . . . a recitation of structural features common to the members of the genus . . . ." *Lilly*, 43 USPQ2d at 1406.

Moreover, proper description of functional features of a claimed invention can also satisfy the written description requirement. In *Enzo Biochem, Inc. v. Gen-Probe Incorporated*, 63 USPQ2d 1609 (Fed. Cir. 2002), the claimed polynucleotide sequences in the patent in question are defined based on their ability to differentially hybridize to reference polynucleotide sequences from deposited bacteria *N. gonorrhoeae* and *N. meningitidis*. The Federal Circuit held that this hybridization function-based description may, in some cases, satisfy the written description requirement because of "a complementary structural relationship" between the claimed sequences and the reference

sequences. *Enzo*, 63 USPQ2d at 1616. The Federal Circuit further stated that "*Lilly* did not hold that all functional descriptions of genetic material necessarily fail as a matter of law to meet the written description requirement; rather, the requirement may be satisfied if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure." *Amgen Inc. v. Hoechst Marion Roussel Inc.*, 65 USPQ2d 1385, 1398 (Fed. Cir. 2003).

2. The Claimed Invention Is Defined by Its Structural and Functional Features

The present invention relates to the identification of nucleic acids encoding a novel G-protein coupled receptor, GPCR-B3. It is Appellants' intent to include in the claim scope nucleic acids encoding allelic variants and man-made muteins that retain the polypeptide's normal function. For example, the specification states that polymorphic variants of rat GPCR-B3 (SEQ ID NO:1) are a part of the invention and provides three substitution variants, *e.g.*, isoleucine substituted by leucine at position 33, aspartic acid substituted by glutamic acid at position 84, and glycine substituted by alanine at position 90 (page 10, lines 11-16, of the specification). The claimed nucleic acids are defined by their shared structural features, *i.e.*, they encode polypeptides with at least 80% amino acid sequence identity to the sequence disclosed in SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

Percent amino acid sequence identity of a polypeptide to a reference amino acid sequence is a structural property of the polypeptide, because such percent identity relies entirely upon the polypeptide's amino acid sequence. This structural attribute of the polypeptide is in turn a structural attribute of the nucleic acid encoding the polypeptide, since the amino acid sequence is determined by the polynucleotide sequence of an encoding nucleic acid. Moreover, the recitation of an amino acid sequence identity makes identification of the polypeptides and thus the claimed nucleic acids easily accomplished by one of skill in the art. Algorithms for determining percent sequence identity and sequence similarity for the identification of polypeptides and their coding

polynucleotide sequences are well known to those of skill in molecular biology and are described in the present specification on pages 19 to 22. The present claims can be analogized with *Fiers*, *Lilly*, and *Enzo* in that they all relate to genetic material. The description of the claimed nucleic acids relies on a percentage sequence identity to a reference sequence and thus establishes a structural feature in a manner even more direct than that in the *Enzo* case.

The claimed nucleic acids are also defined by shared functional features, *i.e.*, they encode polypeptides that are G-protein coupled receptors and bind to glutamate, which induces the GPCR activity. The specification provides functional assays for identifying the polypeptides with such functional features. On page 41, line 27, to page 47, line 10, for instance, the specification teaches isolating a putative GPCR-B3 polypeptide or expressing the polypeptide in a cell, and examining whether the polypeptide is a GPCR protein demonstrating the characteristic GPCR activity, particularly upon binding to a ligand (*e.g.*, glutamate). These assays are used to confirm that a putative GPCR-B3 polypeptide is actually a GPCR polypeptide within the claim scope by examining the polypeptide's physical or chemical effects. The specification further teaches that to demonstrate the GPCR activity, a candidate polypeptide, either naturally occurring or recombinantly produced, can be studied *in vivo* or *in vitro*, when the polypeptide is isolated, expressed in a cell, expressed in a membrane derived from a cell, or expressed in a tissue or in an animal (page 42, lines 13-24). These assays thus allow one skilled in the art to identify the claimed nucleic acids based on the functional attributes of the polypeptides they encode.

Thus, both structural and functional features commonly shared by all members of the claimed genus of GPCR-B3 nucleic acids have been described in detail, which "clearly allow persons of ordinary skill in the art to recognize that [the applicant] invented what is claimed." *Vas-Cath Inc. v. Mahurkar*, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991). This description of the claimed invention is consistent with the holdings of *Lilly*, *Enzo*, and *Amgen*.

In sustaining the claim rejection for alleged inadequate written description, the Examiner contends that the specification does not provide a correlation between any particular structure of the GPCR-B3 polypeptide encoded by the claimed nucleic acid and any particular function, and that reciting a percent sequence identity to a reference sequence does not provide any particular common structure or the information regarding the particular amino acid residues that are important to the GPCR-B3 polypeptide function (the first full paragraph on page 5 of the final Office Action mailed July 13, 2006). Appellants cannot agree with the Examiner.

As discussed above, sequence similarity is a structural feature of a polypeptide and its coding polynucleotide sequence. At the time this application was filed, the inventors clearly had possession of three naturally occurring GPCR-B3 amino acid sequences from rat, mouse, and human (SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively). Relying on sequence alignment and analysis using methods well known to those of skill in the art or methods taught in the present disclosure, an artisan would be able to readily determine whether certain amino acid residues in these proteins are likely to be critical for the protein's functionality, depending on whether these residues are conserved among the three GPCR proteins as well as other known GPCR proteins. Based on this determination, an artisan would be able to derive a large number of amino acid sequences (and their coding polynucleotide sequences) as putative GPCR-B3 polypeptides, which can then be examined for the required functionality according to the methods known in the art or described in the specification. When all these factors are considered, Appellants contend that the three exemplary GPCR-B3 polypeptide sequences not only illustrate their common structural feature but together also provide a reasonably degree of sequence variation in their common structural feature, which is directly tied to the GPCR activity.

3. Summary

Appellants believe that the written description rejection under 35 U.S.C. §112 is improper and should be withdrawn.

VIII. CONCLUSION

In view of the foregoing, Appellants believe all claims now pending in this Application are in condition for allowance.

Respectfully submitted,

Chuan Gao
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60970888 v1

IX. CLAIMS APPENDIX

1. An isolated nucleic acid encoding a taste transduction G-protein coupled receptor, wherein the receptor comprises an amino acid sequence having at least 80% identity to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, and wherein the receptor binds to glutamate, which induces GPCR activity.

4. The isolated nucleic acid of claim 1, wherein the nucleic acid encodes a receptor comprising an amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

5. The isolated nucleic acid sequence of claim 1, wherein the nucleic acid comprises a nucleotide sequence of SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

6. The isolated nucleic acid of claim 1, wherein the nucleic acid is from a human, a mouse, or a rat.

8. The isolated nucleic acid of claim 1, wherein the nucleic acid encodes a receptor having a molecular weight of about between 92 kDa to about 102 kDa.

34. An expression vector comprising the nucleic acid of claim 1.

35. A host cell transfected with the vector of claim 34.

61. A method of making a taste transduction G-protein coupled receptor, the method comprising the step of expressing the receptor from a recombinant expression vector comprising a nucleic acid encoding the receptor, wherein the receptor comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, and wherein the receptor binds glutamate, which induces GPCR activity.

62. A method of making a recombinant cell comprising a taste transduction G-protein coupled receptor, the method comprising the step of transducing the cell with an expression vector comprising a nucleic acid encoding the receptor, wherein the receptor comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, and wherein the receptor binds glutamate, which induces GPCR activity.

63. A method of making an recombinant expression vector comprising a nucleic acid encoding a taste transduction G-protein coupled receptor, the method comprising the step of ligating to an expression vector a nucleic acid encoding the receptor, wherein the receptor comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, and wherein the receptor binds glutamate, which induces GPCR activity.

64. The nucleic acid of claim 1, wherein the receptor comprises an amino acid sequence have at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

65. The method of claim 61, wherein the receptor comprises an amino acid sequence have at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

66. The method of claim 62, wherein the receptor comprises an amino acid sequence have at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

67. The method of claim 63, wherein the receptor comprises an amino acid sequence have at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

X. EVIDENCE APPENDIX

1. Declaration under 37 C.F.R. §1.132 by Dr. Charles Zuker. Submitted September 16, 2004

2. Nelson *et al.*, *Nature*, advance online publication, Feb. 24, 2002 (DOI 10.1038/nature726). Submitted as reference BE in IDS filed September 16, 2004.

XI. RELATED PROCEEDINGS APPENDIX

None.

X. EVIDENCE APPENDIX

1. Zuker Declaration

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail Label No. EL 951985842 US in an envelope addressed to:

Assistant Commissioner for Patents
Washington, D.C. 20231

On September 16, 2002

TOWNSEND and TOWNSEND and CREW LLP

By: Dana Kane

PATENT
Attorney Docket No.: 02307E-088610
Client Ref. No. UC 98-306-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Zuker *et al.*

Application No.: 09/361,652

Filed: July 27, 1999

For: **NUCLEIC ACIDS ENCODING A G-PROTEIN COUPLED RECEPTOR INVOLVED IN SENSORY TRANSDUCTION**

Examiner: Michael Brannock

Art Unit: 1646

DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. CHARLES ZUKER

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Charles Zuker, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I received my Ph.D. from Massachusetts Institute of Technology. I am currently a Professor and Investigator, Howard Hughes Medical Institute, Departments of Biology and Neurosciences, School of Medicine, University of California at San Diego. I have been in this position since 1986. See resume, Exhibit A.

4. The above-referenced patent application claims isolated nucleic acids encoding GPCR-B3, also known as T1R1, a taste bud specific G protein coupled receptor involved in taste transduction.

5. I am an inventor of the above-referenced patent application. I have read and am familiar with the contents of the patent application. In addition, I have read the Office Action, mailed August 12, 2001, received in the present case. It is my understanding that the Examiner believes that this invention is supported by neither a specific, substantial, and credible asserted utility nor a well established utility as required by the United States Patent Laws.

6. This declaration is provided to demonstrate that, at the time the application was filed, one of skill in the art would recognize the utility of the present invention and would appreciate its real world context.

7. The present application discloses that the claimed nucleic acid, a full length cDNA, encodes a G protein coupled receptor ("GPCR") that is specifically expressed in taste buds of the tongue, and provides data demonstrating that the claimed protein is a functional G-protein coupled receptor. The present invention is therefore useful, e.g., for screening for taste modulators of a taste bud cell specific GPCR, for the identification of GPCR-B3 taste ligands, and as a specific marker for specialized taste bud cells of the tongue.

8. As described in the present specification, full length cDNAs that encode a taste cell-specific nucleic acids were cloned. Sequence analysis of the GPCR-B3 clone showed that it had the structure of a G-protein coupled receptor, with an extracellular domain, seven transmembrane domains, and a cytoplasmic domain (*see, e.g.*, Example I, page 56-57). Subsequently, protein expression patterns were determined for GPCR-B3 using *in situ* analysis (*see, e.g.*, Example II, page 58, and Figure 3). Figure 3 shows that the claimed nucleic acids express proteins that are specifically expressed in taste buds of the tongue.

9. Furthermore, the specification provides experimental data demonstrating that GPCR-B3 is a functional G-protein coupled receptor. Figure 4 shows the structure of a chimeric protein, comprising an extracellular domain of a murine MGluR1 receptor fused to the seven transmembrane domains and cytoplasmic domains of GPCR-B3. This chimeric GPCR construct was transfected into HEK cells, which were then stimulated with glutamate, the MGluR1 ligand. The HEK cells demonstrated an increase in intracellular calcium in response to the ligand, indicating that the chimeric GPCR couples to a promiscuous G protein and triggers calcium responses that are detectable using the indicator fura-2. The presently claimed GPCR-B3 nucleic acids therefore encode a G protein coupled receptor that is specifically expressed in fungiform and foliate cells of the tongue, which are taste bud cells, as described in the specification.

10. It would be apparent to anyone of skill in the art that GPCR-B3 is an excellent target for candidate compounds that modulate taste transduction. This use is not merely a "starting point for further research and investigation," but a direct assay for taste ligands and modulators of taste signal transduction. Furthermore, the claimed nucleic acids are specifically expressed in a unique subset of tongue cells, and the encoded proteins localize to the taste pore- the subcellular location for taste receptors. As such, they have specific and substantial utility as markers for specialized taste cells of the tongue. Such markers are useful for the generation of taste topographic maps the

elucidate the relationship between taste bud cells of the tongue and taste sensory neurons leading to taste centers in the brain. Applicants have therefore provided a nucleic acid that encodes a protein with known signaling activity and specific expression in a specialized sub-set of cells.

11. In view of the foregoing, it is my scientific opinion that one of skill in the art, at the time the application was filed, would immediately recognize the real world utility of the nucleic acids of this invention. Therefore, this invention is supported by a specific, substantial, and credible utility.

Date: 9/10/02 By: _____

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EDUCATION

INSTITUTION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Universidad Catolica de Valparaiso; Chile	B.Sc., Honors	1977	Biology
Massachusetts Inst. of Technology; Boston	Ph.D.	1983	Biology

RESEARCH AND/OR PROFESSIONAL EXPERIENCE

1993 - present	Professor and Investigator; Howard Hughes Medical Institute Departments of Biology and Neurosciences, School of Medicine University of California, San Diego
1989 - 1992	Associate Professor and Associate Investigator Howard Hughes Medical Institute, UCSD
1986 - 1989	Assistant Professor; Department of Biology, UCSD
1983 - 1986	Postdoctoral Fellow; Department of Biochemistry; University of California, Berkeley
1977 - 1983	Graduate Student; Department of Biology; Massachusetts Institute of Technology

Honors and Keynote Lectures (selected)

Whitaker Health Sciences Fund Fellow, Massachusetts Inst. of Technology, 1979-1980
Whitaker Health Sciences Fund Fellow, Massachusetts Inst. of Technology, 1981-1982
European Molecular Biology Organization Fellow, 1983
Jane Coffin Childs Memorial Fund for Medical Research Fellow, 1984-1986
McKnight Foundation Fund for Neuroscience Award, 1988-1991
Monsanto Speaker, St. Louis University, St Louis, MO, 1991
Broadhurst Foundation visiting lecturer, Cambridge, MA, 1991
Institute Speaker, Scripps Research Institute, La Jolla, CA, 1992
Keynote speaker, Stanford Neurosciences Program Retreat, Monterey, CA,1992
Pew Scholars Award, 1988-1992
Alfred P. Sloan Award in Neurosciences, 1988-1990
March of Dimes Basil O'Connor Award, 1989-1991
Merck Lecturer, UC Berkeley 1992
Institute speaker, Roche Institute of Molecular Biology, Nutley, NJ, 1993
Keynote Speaker, Pharmacological Sciences Program, Vanderbilt University, Nashville, TN, 1994
Keynote Speaker, Stanford Medical Scientist Training Program, Stanford University CA, 1994
Lecturer in the Life Sciences, Northwestern University Medical School, Chicago, IL 1994

Howard Hughes Medical Institute, Lecture series to Institute employees, Howard Hughes Medical Institute, Chevy Chase, MD, 1996
Keynote Speaker, FASEB Summer Conference on "The Biology and Chemistry of Vision", Keystone, CO, 1997
Keynote Speaker, U. Penn Graduate programs in Biochemistry, Molecular Biology and Pharmacology. Philadelphia, 1998
Cogan Award, Association for Research in Vision and Ophthalmology, 1998
University Lecturer, UT Southwestern Medical School, 1999
Alcon Award for outstanding contributions to vision research, 1999
American Academy of Arts and Sciences, 2000

Study Sections and Advisory Boards (selected):
Member, Scientific Advisory Board, Pew Latin American Scholars Program, 1990 - present
Mechanisms of Development, 1991-present
Neuron, 1995-present
Member, American Cancer Society Postdoctoral Research Selection Committee, 1995-1999
Member, Scientific Advisory Board, Schepens Research Institute, Harvard University, Cambridge, MA, 1995 - present
Member, Review Panel, Howard Hughes Medical Institute International Grants Program, 1996
Member, National Research Council/ National Academy of Sciences advisory committee for the US and HHMI program in Latin America, 1997-
National Advisory Committee of The Pew Scholars Program in the Biomedical Sciences, 1997-
Member, NIH Visual Sciences C study section, Bethesda, MD, 1997-2000
Member, NIDCD Strategic Planning committee 1999-
Damon Runyon-Walter Winchell Cancer Fund Scientific Advisory Committee, 1999-
Current Biology, 2000-
Steering Committee, Alliance for Cellular Signaling, 2000-
Advisory board, Pew program in Science and Society, 2001-
Advisory board, NIH-wide initiative on mouse mutagenesis, 2001-

Publications (selected):

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X. EVIDENCE APPENDIX

2. Nelson *et al.* reference

An amino-acid taste receptor

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The sense of taste provides animals with valuable information about the nature and quality of food. Mammals can recognize and respond to a diverse repertoire of chemical entities, including sugars, salts, acids and a wide range of toxic substances[1]. Several amino acids taste sweet or delicious (umami) to humans, and are attractive to rodents and other animals[2]. This is noteworthy because L-amino acids function as the building blocks of proteins, as biosynthetic precursors of many biologically relevant small molecules, and as metabolic fuel. Thus, having a taste pathway dedicated to their detection probably had significant evolutionary implications. Here we identify and characterize a mammalian amino-acid taste receptor. This receptor, T1R1+3, is a heteromer of the taste-specific T1R1 and T1R3 G-protein-coupled receptors. We demonstrate that T1R1 and T1R3 combine to function as a broadly tuned L-amino-acid sensor responding to most of the 20 standard amino acids, but not to their D-enantiomers or other compounds. We also show that sequence differences in T1R receptors within and between species (human and mouse) can significantly influence the selectivity and specificity of taste responses.

T1Rs and T2Rs are two families of G-protein-coupled receptors (GPCRs) selectively expressed in subsets of taste receptor cells[3–11]. T2Rs are involved in bitter taste detection[4,5], and T1R2 and T1R3 combine to function as a sweet taste receptor[7]. To identify taste receptors involved in amino-acid detection, we used an expression screening strategy similar to that used in the characterization of bitter and sweet taste receptors. Candidate receptors were expressed in human embryonic kidney (HEK) cells containing the $G\alpha_{16}$–$G\alpha_z$ and $G\alpha_{15}$ promiscuous G proteins[12,13], and assayed for stimulus-evoked changes in intracellular calcium. In this system, receptor activation leads to activation of phospholipase Cβ (PLC-β) and release of calcium from internal stores, which can be monitored at the single-cell level using calcium-indicator dyes[5,7,14].

Because T1R taste receptors are distantly related to GPCRs that recognize the amino acids glutamate[15] (metabotropic glutamate receptors, mGluRs), GABA[16] (γ-aminobutyric acid; GABA-B receptors) and arginine[17] (the R5-24 receptor), we began by testing members of the T1R family. Patterns of T1R expression define at least three distinct cell types: cells co-expressing T1R2 and T1R3 (T1R2+3, a sweet receptor), cells co-expressing T1R1 and T1R3 (T1R1+3) and cells expressing T1R3 alone[7]. First, we assayed responses of the T1R2+3 sweet taste receptor to all 20 standard and various D-amino acids. Several D-amino acids that taste sweet to humans, and are attractive to mice, trigger robust activation of the T1R2+3 sweet taste receptor (Fig. 1a, b). However, none of the tested L-amino acids activate this receptor.

Mouse T1R1 and T1R3 were transfected alone or in combination and tested for stimulation by L-amino acids. Individual receptors showed no responses. In contrast, T1R1 and T1R3 combine to function as a broadly tuned L-amino-acid receptor, with most amino acids that are perceived as sweet (for example, alanine, glutamine, serine, threonine and glycine[2]) activating T1R1+3 (Fig. 1). The responses are strictly dependent on the combined presence of T1R1 and T1R3, and are highly selective for L-amino acids; D-amino acids and other natural and artificial sweeteners did not activate the T1R1+3 receptor combination. These results substantiate T1R1+3 as a receptor for L-amino acids, and provide a striking example of heteromeric GPCR receptors radically altering their selectivity by a combinatorial arrangement of subunits.

If T1R1+3 functions as a major L-amino acid taste sensor *in vivo*, we might expect its cell-based behaviour to recapitulate some of the physiological properties of the *in vivo* receptor. Nerve recordings in rats have shown that taste responses to L-amino acids are considerably potentiated by purine nucleotides such as inosine monophosphate (IMP)[18]. To assay the effect of IMP, HEK cells expressing the T1R1+3 receptor combination were stimulated with amino acids in the presence or absence of IMP. Indeed, T1R1+3 responses to nearly all L-amino acids were dramatically enhanced by low doses of IMP (Figs 1 and 2a); this effect increased over a range of 0.1–10 mM (Fig. 2b). However, IMP alone elicited no response, even at the highest concentration tested in our assays, and it had no effect on responses mediated by T1R2+3 (either to sweeteners or to L- and D-amino acids; data not shown).

T1R1+3 is prominently expressed in fungiform taste buds[7], which are innervated by chorda tympani fibres. Therefore, we stimulated mouse fungiform papillae at the front of the tongue with various amino acids in the presence or absence of IMP, and recorded tastant-induced spikes from the chorda tympani nerve. As expected, nerve responses to L-amino acids were significantly enhanced by IMP[18] (Fig. 3). However, IMP had no significant effect on responses to D-amino acids or to non-amino-acid stimuli.

Genetic studies of sweet tasting have identified a single principal locus in mice influencing responses to several sweet substances (the *Sac* locus[19,20]). Sac 'taster' mice are about fivefold more sensitive to sucrose, saccharin and other sweeteners than Sac non-tasters. *Sac* codes for T1R3[7–11,21]. There are two amino-acid differences that define taster and non-taster alleles[7,9,10]. One of these changes, I60T, introduces a potential glycosylation site that was proposed to eliminate receptor function by preventing receptor dimerization[10]. This poses a conundrum because responses to L-amino acids are not influenced by the *Sac* locus[7,22] (and data not shown). Thus, if T1R3 functions as the common partner of the sweet and amino-acid receptors, we reasoned that the T1R3 non-taster allele must selectively affect the T1R2+3 combination.

We examined the effect of the *Sac* non-taster allele on T1R1 and T1R2 using biochemical and functional assays. First, we investigated receptor heteromerization by co-immunoprecipitating differentially tagged T1R receptors. In essence, HEK cells were co-transfected with taster and non-taster alleles of T1R3 and either haemagglutinin (HA)-tagged T1R1 or T1R2. Receptor complexes were then immunoprecipitated with anti-HA antibodies, and the association with T1R3 assayed with anti-T1R3 antibodies. Other results demonstrated that the non-taster form of T1R3, much like its taster counterpart, assembles into heteromeric receptors with T1R1 and T1R2 (Fig. 4a). This argues against the possibility that the sweet taste deficits of Sac non-taster animals result from failure to assemble heteromeric receptors. Second, we examined the functional responses of T1R2+3 (sweet) and T1R1+3 (amino acid) receptors carrying either the taster or non-taster allele of T1R3. The taster and non-taster alleles of T1R3 generate functionally similar receptors when combined with T1R1, but the non-taster form displays significantly impaired responses when combined with T1R2 (Fig. 4b). Thus, responses to L-amino acids are not affected by the *Sac* locus in mice because *Sac* selectively affects the T1R2+3 receptor combination.

The finding that polymorphism in one of the T1R receptor

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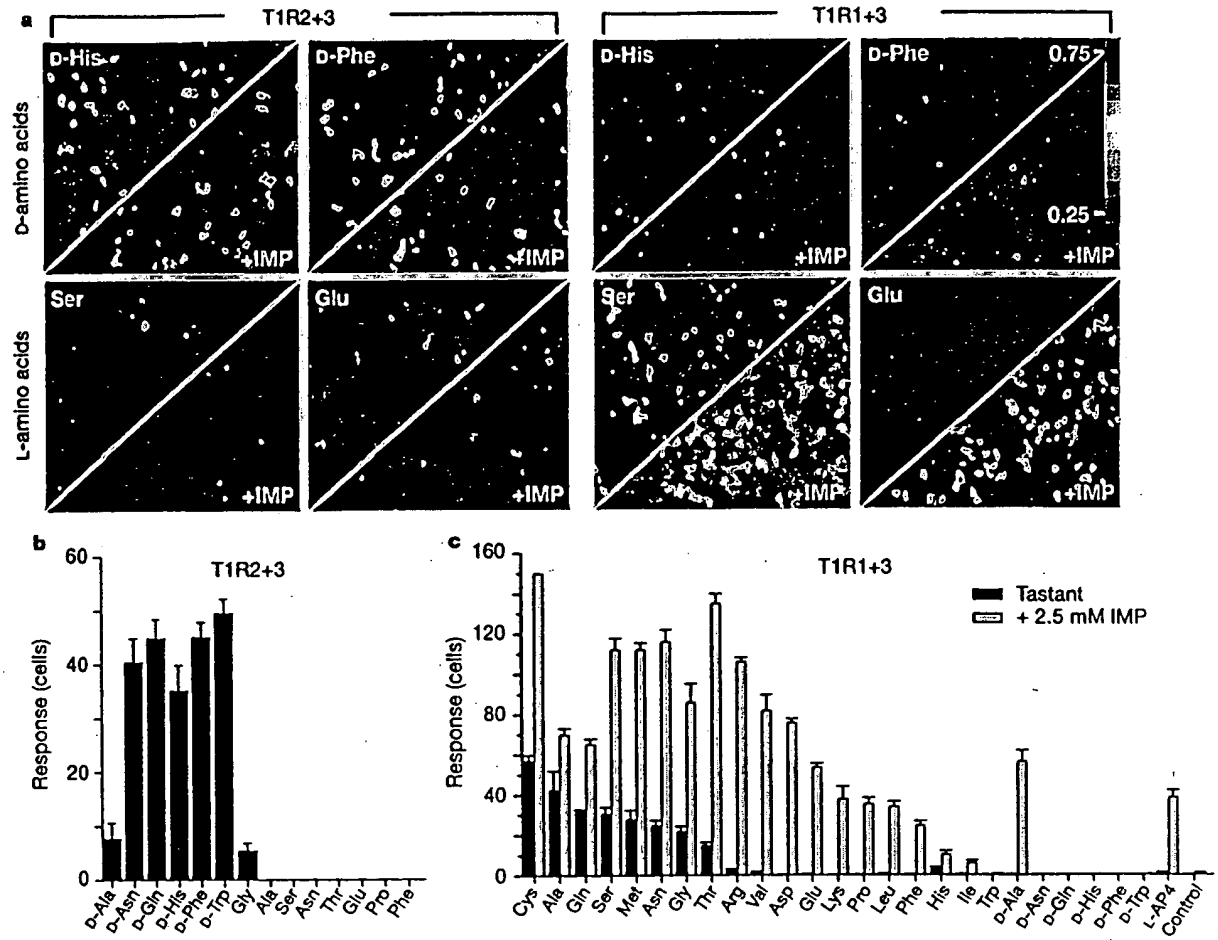

Figure 1 T1R receptor combinations respond differentially to L- and D-amino acids. **a**, HEK-293 cells co-expressing promiscuous G proteins and heteromeric mouse T1R2+3 or T1R1+3 receptors were stimulated with L- and D-amino acids. The T1R2+3 sweet taste receptor is activated by sweet-tasting D-amino acids but not by L-amino acids (left). In contrast, T1R1+3 is activated by L-amino acids and responses are potentiated by IMP (right). Amino acids were 50 mM and IMP was 2.5 mM; the colour scale indicates the F_{340}/F_{380} ratio (see Methods). **b**, **c**, Quantification of amino-acid responses for T1R2+3 (b) and T1R1+3 (c). Amino acids were 50 mM, and IMP and L-AP4 were 2.5 mM; control refers to 2.5 mM IMP alone. Each column represents the mean ± s.e.m. of at least ten independent determinations. IMP had no effect on T1R2+3 (data not shown). D-Amino acids (with the exception of D-Ala in the presence of IMP) and natural or artificial sweeteners did not activate T1R1+3. Trp elicited no responses and Tyr was not assayed because it is insoluble at high concentration. Note that the achiral amino acid Gly activates both receptor complexes. All calcium measurements and quantifications were performed as described in the Methods and ref. 7.

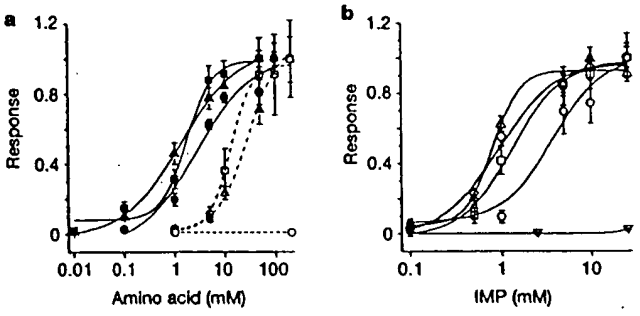

Figure 2 Dose response of T1R1+3 to L-amino acids and IMP. **a**, Dashed lines with open symbols represent dose responses of T1R1+3 with L-amino acids (squares, Ala; circles, Glu; triangles, Ser). The presence of 2.5 mM IMP (solid lines with filled symbols) shifts the responses by at least one order of magnitude to the left. Equivalent results were obtained with most L-amino acids (see also Fig. 1b). **b**, IMP potentiates responses of T1R1+3. Shown are dose responses for Ala (2 mM, squares), Glu (4 mM, circles), Ser (2 mM, triangles), Gly (4 mM, diamonds) and IMP (inverted triangles). Responses were normalized to the mean response at the highest concentration. Each point represents the mean ± s.e.m of at least ten assays.

subunits differentially affects receptor function suggests that other sequence variations in the amino-acid and sweet receptors may significantly influence tastant sensitivity or selectivity. For example, humans can taste a number of artificial sweeteners that rodents cannot (for example, aspartame, cyclamate and various sweet proteins[23]). Rodent and human T1Rs are only about 70% identical[7]. Therefore, we generated heteromeric receptors consisting of human and rodent T1R subunits and assayed for activation by amino acids and artificial sweeteners. Indeed, the presence of human T1R1 or T1R2 greatly altered the sensitivity (Fig. 4c) and the specificity (Fig. 4d) of the amino acid sweet receptors. Cells expressing human T1R1 are more than an order of magnitude more sensitive to glutamate than to other amino acids, and cells expressing human T1R2 robustly respond to aspartame, cyclamate and intensely sweet proteins (Fig. 4d and data not shown). Thus, the nature of the unique partner determines whether the receptor complex will function as a sweet receptor or as an amino-acid receptor, and sequence differences in T1Rs between or within species (for example, polymorphisms in *Sac*) can greatly influence taste perception.

In humans, monosodium L-glutamate (MSG) elicits a unique

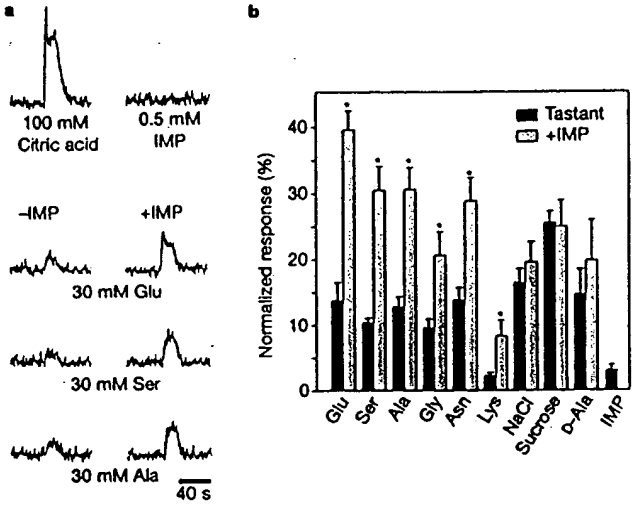

Figure 3 IMP stimulates responses of the chorda tympani nerve to amino acids in mice. **a**, Integrated neural responses of C57BL/6 mice to Glu, Ser and Ala (30 mM each) were recorded with and without 0.5 mM IMP. The responses to 100 mM citric acid and 0.5 mM IMP alone are shown in the upper traces. Equivalent results were obtained for most L-amino acids. **b**, Integrated neural responses, such as those shown in **a**, were normalized to the responses of 100 mM citric acid. Black bars, tastant alone; grey bars, tastant + 0.5 mM IMP. The values are means ± s.e.m. ($n = 5$). Sucrose was used at 100 mM and all other tastants at 30 mM. Asterisks indicate statistically significant differences ($P < 0.05$).

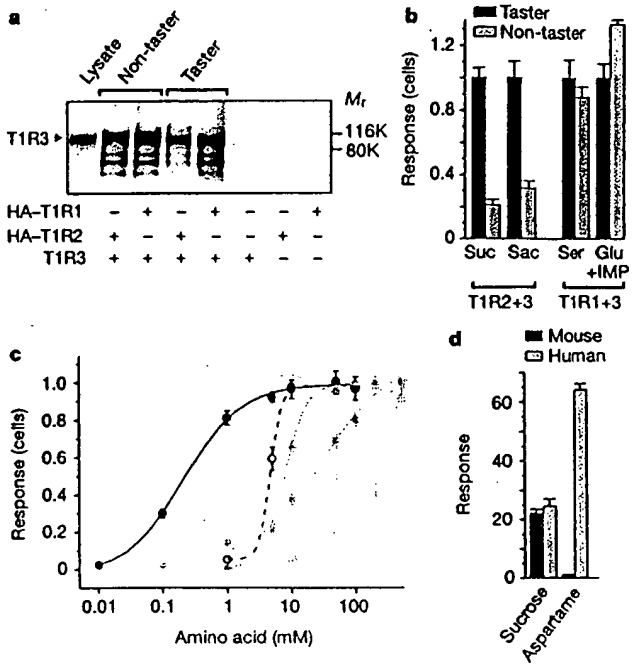

Figure 4 Polymorphic differences in T1Rs influence receptor function. **a**, Immunoprecipitation and western blot analyses shows that *Sac* non-taster and taster alleles of T1R3 form heteromeric complexes with T1R1 and T1R2. Cells were transfected with combinations of T1Rs as indicated. All extracts were immunoprecipitated with anti-HA antibodies, and the resulting protein complexes probed with anti-T1R3 antibodies. M_r, relative molecular mass, in thousands (K). **b**–**d**, Results of cell-based calcium imaging assays. **b**, The *Sac* allele selectively affects the T1R2+3 heteromeric receptor. Responses were normalized to the mean responses obtained with the taster allele (black bars). The responses of T1R2+3 to sweet compounds are significantly reduced when the non-taster T1R3 allele is used, but responses of T1R1+3 to amino acids are unaffected, even in the presence of IMP. **c**, Human T1R1 influences sensitivity to monosodium L-glutamate. Low-concentration MSG robustly activates receptors containing human T1R1 (open circles), and IMP potentiates the response (filled circles). Also shown for comparison are dose responses for Ala (squares) and Ser (triangles). For each series, responses were normalized to the mean response at the highest concentration. **d**, Mouse T1R2+3 (black bars) responds to sucrose and other natural and artificial sweeteners, but not aspartame. However, substituting human T1R2 for mouse T1R2 (grey bars) in the rodent T1R2+3 receptor imparts aspartame sensitivity.

savoury taste sensation called umami[24,25]. Hallmarks of the umami taste are its potentiation by purine nucleotides, and activation by the mGluR-agonist L-AP4 (ref. 25). A mGluR4 splice variant has recently been isolated as a candidate umami receptor[26]. An important question is whether T1R1+3 is umami receptor. Our results demonstrate that T1R1 and T1R3 combine to function as a broadly tuned amino-acid receptor. Notably, T1R1+3 responses to L-AP4 (Fig. 1), MSG and other amino acids are greatly potentiated by purine nucleotides. Thus, we propose that T1R1+3 is a constituent of the umami response. Future studies should help define whether T1R1+3 is the principal, or an additional, umami receptor. An interesting paradox that emerged from this work is the relationship between receptor activity and taste perception. For example, T1R1+3 responds to most L-amino acids, but not all amino acids taste the same: some are attractive to mice and sweet to humans, whereas others are neutral; some are even perceived as bitter and are aversive to animals[2]. Similarly, very few amino acids elicit the taste of umami. The identification of bitter, sweet, and now an amino-acid taste receptor provide a powerful platform to help decode the interplay between the various taste modalities, and the link between events at the periphery (taste receptor cells) and the central nervous system (perception and behaviour). □

Methods

Heterologous expression and calcium imaging

Cells were grown, maintained and transfected exactly as described earlier[7]. Transfection efficiencies were estimated by co-transfection with a green fluorescent protein (GFP) reporter plasmid and were typically >70%. FURA-2 acetomethyl ester was used to measure intracellular calcium concentration ($[Ca^{2+}]_i$), and assay conditions were identical to those previously described[7]. Responses were measured for 60 s and the fluorescence ratio at wavelengths of 340 and 380 nm (F_{340}/F_{380}) was used to measure $[Ca^{2+}]_i$. For data analysis, response refers to the number of cells responding in a field of about 300 transfected cells. Cells were counted as responders if F_{340}/F_{380} increased above 0.27 after addition of tastant. In general, >90% of the responding cells had $F_{340}/F_{380} > 0.35$. Dose-response functions were fitted using the logistical equation. Studies involving taster and non-taster alleles of T1R3 used contructs of complementary DNA coding for T1R3 from C57BL/6 and 129/Sv mice, respectively[7–11,21].

Immunoprecipitation

Antibodies against T1R3 were generated using a peptide corresponding to residues 824–845 of the mouse receptor. PEAK^{rapid} cells (Edge Biosciences) were transfected with HA–T1R1, HA–T1R2 and T1R3 in various combinations and were gathered and disrupted in buffer containing 50 mM Tris-HCl at pH 7.5, 300 mM NaCl, 1% NP-40, 0.5% w/v sodium deoxycholate, and protease inhibitors (Roche). Lysates were incubated overnight at 4 °C with mouse monoclonal anti-HA antibody (Santa Cruz) and immune complexes were collected with protein AG–agarose beads. Samples were fractionated by SDS–PAGE, transferred to nitrocellulose membrane and probed with anti-T1R3 antibody. As a control for the specificity of the interactions, we have shown that artificially mixing extracts from cells expressing tagged T1R1 or T1R2 with extracts from cells expressing T1R3 does not produce complexes. Similarly, co-transfection of a Rho-tagged mGluR1 receptor[15] did not produce T1R–GluR1 complexes.

Nerve recording

Lingual stimulation and recording procedures were performed as previously described[27]. Neural signals were amplified (2,000×) with a Grass P511 AC amplifier (Astro-Med), digitized with a Digidata 1200B A/D convertor (Axon Instruments), and integrated (r.m.s. voltage) with a time constant of 0.5 s. Taste stimuli were presented at a constant flow rate of 4 ml min^{-1} for 20-s intervals interspersed by 2-min rinses between presentations. All data analyses used the integrated response over a 25-s period immediately after the application of the stimulus. Each experimental series consisted of the application of six tastants bracketed by presentations of 0.1 M citric acid to ensure the stability of the recording. The mean response to 0.1 M citric acid was used to normalize responses to each experimental series.

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Competing interests statement

The authors declare that they have no competing financial interests.

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